Genetic diversity of *Rhagada* land snails on Barrow Island

Michael S. Johnson[1], Zoë R. Hamilton[1,2] & Jeremy Fitzpatrick[3]

[1] School of Animal Biology (M092), University of Western Australia, Crawley, WA 6009.
✉ msj@cyllene.uwa.edu.au
[2] Biota Environmental Sciences Pty Ltd, PO Box 155, Leederville, WA 6903.
✉ zoe@biota.net.au
[3] RPS Bowman Bishaw Gorham, 290 Churchill Avenue, Subiaco, WA 6008.
✉ jfitzpatrick@rpsbbg.net.au

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Abstract

The dominant group of land snails in the Pilbara Region is the camaenid genus *Rhagada*, which includes several species confined to islands. Analysis of allozymes confirmed the presence of two genetically distinct species of *Rhagada* on Barrow Island: a small species restricted to the northern tip of the island, and a large species widespread over the remainder of the island. Comparisons amongst 19 samples of the widespread, large species revealed distinct populations, but with unusually low levels of genetic subdivision, and no detectable geographic pattern on the island. In contrast with the low divergence within species, the two species are genetically the most distinctive of all species of *Rhagada* examined from the Pilbara Region. The genetic distinctiveness of these species highlights the conservation value of Barrow Island for these endemic snails, and raises questions of the evolutionary history of *Rhagada* in the Pilbara Region.

Keywords: *Rhagada*, genetic diversity, land snails, Barrow Island

Introduction

Islands are of special interest in the context of evolution and, increasingly, conservation. Genetic studies have found divergent populations of mammals (*e.g.*, Schmitt 1978; Moro *et al.* 1998; Eldridge *et al.* 1999; Sinclair 2001; Hinten *et al.* 2003) and reptiles (*e.g.*, Sarre *et al.* 1990) on isolated Australian islands. In addition to facilitating genetic divergence, islands are important refugia and play a special role for conservation of terrestrial fauna in Western Australia.

In both evolutionary and conservation contexts, there has been a strong focus on vertebrate species. Terrestrial invertebrates, on the other hand, often have less capacity for dispersal and hence smaller geographic distributions than species of vertebrates (*e.g.*, Harvey 2002). Limited dispersal and narrow distributions increase both the likelihood of locally distinct genetic forms and their vulnerability to extinction. Land snails are well recognised for their limited vagility and often small distributions, but have been poorly studied compared with vertebrates (*e.g.*, Ponder 1997). To illustrate, Ponder (1997) pointed out that there are more than 900 species of terrestrial molluscs in Australia, with the actual total probably being closer to 2000, but he could find only three genetic studies of native species (Hill *et al.* 1983; Woodruff & Solem 1990; Daniell 1994). We know of only four subsequent genetic studies (Clarke & Richardson 2004; Hugall *et al.* 2002, 2003; Johnson *et al.* 2004).

In the Pilbara Region, the dominant group of land snails is the genus *Rhagada*, which is endemic to northern Western Australia (Solem 1997). Although mainland species in the Pilbara Region tend be distributed over hundreds of kilometres, there are unique species restricted to islands in the Dampier Archipelago and the Montebello Islands group (Solem 1997). There appear to be two species of *Rhagada* on Barrow Island (Slack-Smith 2002), the smaller species being approximately 10 mm diameter and the larger species approximately 20 mm diameter (Fig. 1). These species have not been taxonomically described or assigned to any species. The small species has been found only on the northern end of Barrow Island (Slack Smith 2002), although its shells closely resemble those of *R. plicata*, which Solem (1997) reported from the nearby Montebello Islands. The larger species is abundant and widespread over the rest of Barrow Island. In their physiological study, Withers *et al.* (1997) referred to this latter species as *R. tescorum*, a mainland species, but its similarity to both mainland *R. capensis* and *R. convicta* and to the Dampier Archipelago species *R. perprima* emphasises the taxonomic difficulties of this genus (Slack-Smith 2002).

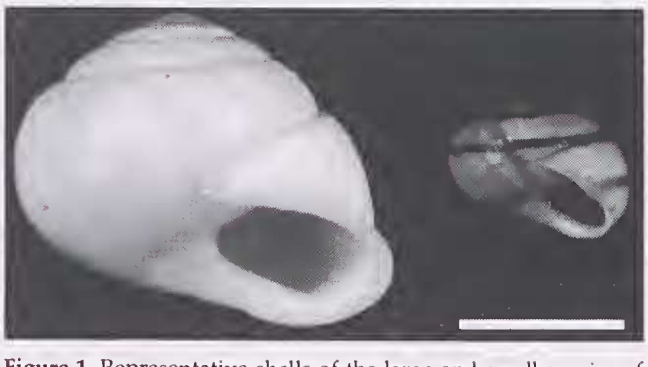

Figure 1. Representative shells of the large and small species of *Rhagada* on Barrow Island. Scale bar = 1 cm.

In this study, allozyme electrophoresis was used to examine genetic diversity of *Rhagada* on Barrow Island, to answer questions at four levels:

(a) Are the two apparent (morphologically distinct) species on Barrow Island genetically distinct? Snail shells can vary greatly among populations of the same species (*e.g.*, Johnson *et al.* 1993; Johnson & Black 2000). Consequently, shell morphology alone may not be reliable for differentiating the two species of *Rhagada*. Independent genetic evidence for species distinctness is, therefore, important for evaluating the relationships of the small and large forms.

(b) Are the species on Barrow Island genetically unique? Unusually high levels of genetic similarity have been found among species of *Rhagada* on the mainland and in the Dampier Archipelago (Johnson *et al.* 2004). Comparison of the species on Barrow Island with those from other parts of the Pilbara Region will determine whether the island species are unique.

(c) How much genetic divergence is there among populations of the widespread, larger species on Barrow Island and what is the geographic pattern of that divergence?

(d) At the smaller scale, are there genetically distinct groups of populations?

This is the first study of local genetic subdivision within a species of *Rhagada*.

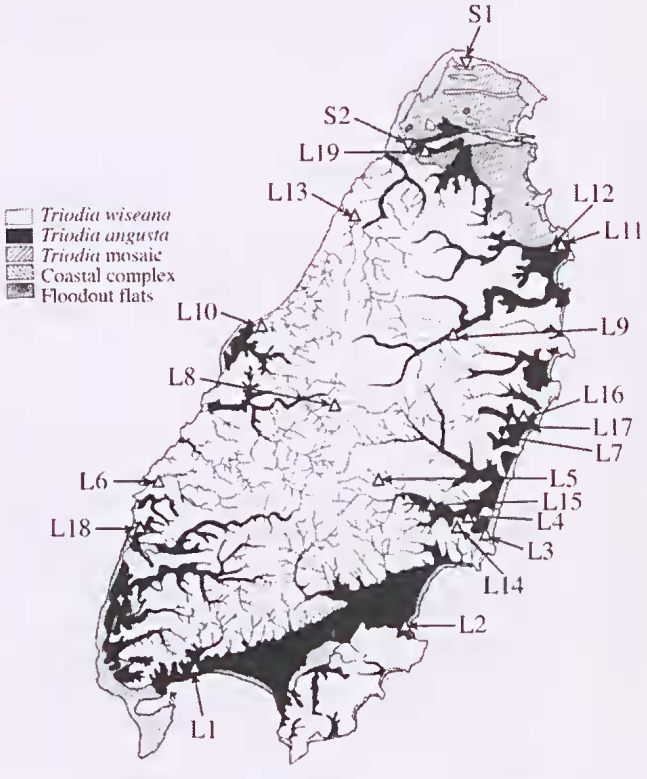

Figure 2. Sample sites of *Rhagada* on Barrow Island. L = large species; S = small species. Vegetation map from Buckley (1983).

Methods

Samples

Live, adult *Rhagada* were collected between March and July 2004, at 21 sites across Barrow Island, providing a good coverage of the island (Fig. 2). Two samples of the small species were collected from the northern portion of the island, and 19 samples of the large species were collected from widely dispersed sites across the island. Sampling sites are superimposed on the vegetation map of Buckley (1983) in Figure 2.

In the laboratory, the snails were activated overnight, by placing them on moist tissue paper in sealed plastic boxes, and were then frozen at -80°C, pending allozyme electrophoresis. This ensured that the processed snails were alive and active.

Allozyme electrophoresis

Preparation of samples and allozyme electrophoresis followed the procedures used in a previous study of *Rhagada* from the Pilbara mainland and Dampier Archipelago (Johnson *et al.* 2004). Thirteen enzymes, representing 15 gene loci, were successfully examined: adenylate kinase (EC 2.7.4.3; *Ak* locus); arginine phosphokinase (EC 2.7.3.3; *Apk*); glucosephosphate isomerase (EC 5.3.1.9; *Gpi*); isocitrate dehydrogenase (EC 1.1.1.42; *Idh1* and *Idh2*); lactate dehydrogenase (EC 1.1.1.27; *Ldh*); leucine amino peptidase (EC 3.4.-.-; *Lap*); leucyl-glycylglycine peptidase (EC 3.4.-.-; *Pep-lgg*; TEB); leucyl-leucine peptidase (EC 3.4.-.-; *Pep-ll*); leucyl-tyrosine peptidase (EC 3.4.-.-; *Pep-lt*); valyl-leucine peptidase (EC 3.4.-.-; *Pep-vl*); phosphoglucomutase (EC 5.4.2.2; *Pgm1* and *Pgm2*); phosphogluconate dehydrogenase (EC 1.1.1.43; *Pgd*); and superoxide dismutase (EC 1.15.1.1; *Sod*). Multiple loci for a particular enzyme and allozymes at each locus were labelled according to relative electrophoretic mobility.

Samples were processed in two stages. First, four snails from each site were examined for all fifteen loci. Both the large and the small species were included, providing genetic comparison between the two apparent species on Barrow Island. In addition, a sample of *Rhagada convicta* (the most widspread species on the mainland) from Mundabullagana Station (20° 08' 09.7" S, 118° 01' 31.0" E) was included. Because the species examined so far are all very similar for their allozymes, this sample provided an adequate link to the published genetic comparisons among all known species of *Rhagada* from the Pilbara mainland and most of the species from the Dampier Archipelago (Johnson *et al.* 2004), placing the species from Barrow Island in the broader geographic context.

In addition to determining whether the species on Barrow Island are genetically distinct, this first stage of electrophoresis determined which enzymes were genetically variable in the large, widespread species on Barrow Island. These variable enzymes were then examined in larger samples of this species from all 19 sites. Five sites (L14-17, L19 in Fig. 1) were represented by small samples (10–11 snails), while the other 14 sites had more reliable samples of 33 to 52 snails.

Analysis of data

Allelic frequencies were calculated at each locus, and differences between populations were measured over all 15 loci as Nei's (1978) genetic distance. The matrix of

genetic distances was summarized by a UPGMA phenogram, using PHYLIP version 3.64 (Felsenstein 1993). The phenogram was illustrated with the help of TreeView (Page 1996). This analysis included all 21 samples from Barrow Island and the species of *Rhagada* from the Pilbara mainland and the Dampier Archipelago (Johnson *et al.* 2004).

For the variable loci in the large species on Barrow Island, genetic subdivision among all 19 samples was measured as Wright's fixation index, F_{ST}, using GENEPOP (Raymond & Rousset 1995), as implemented on the web (http://biomed.curtin.edu.au/genepop). F_{ST} is the proportion of genetic variation due to differences between populations. The statistical significance of genetic differences among populations was tested by randomization contingency tests, using GENEPOP. Differences between pairs of sites were measured as pairwise F_{ST}. Pairwise F_{ST} was also plotted against geographic distance between sites, to determine whether there was a pattern of isolation by distance. The significance of the association of genetic distance with geographic distance was tested with a Mantel test, using GENEPOP.

Results

Comparisons between species

The genetic comparisons confirmed the distinctness of the large and small species of *Rhagada* on Barrow Island. The two species are completely different at the *Ldh*, *Pgd* and *Pgm1* loci and have very divergent frequencies of alleles at the *Gpi*, *Idh1* and *Pep-vl* loci (Table 1). The average genetic distance between the two species was 0.34. Although not unusually divergent for congeneric species, the Barrow Island species are genetically the most distinctive of those tested so far in this region. The phenogram illustrates the distinctness of the Barrow Island *Rhagada* from the species on the Pilbara mainland and the Dampier Archipelago (Fig. 3). The large species on Barrow Island clusters with the group of species from the mainland and the Dampier Archipelago, but is distinguished by fixation of the unique Ldh^{111} allele. The small species is well separated from all the other species, in its own cluster.

Spatial variation within the large species

The phenogram also illustrates the high degree of similarity of populations within each of the species on Barrow Island. Further genetic analysis was conducted on the widespread, large species, to determine the amount and pattern of genetic divergence across the island. Only three of the 15 allozyme loci had multiple alleles in this species. At two of these (*Gpi* and *Pgm1*), one allele predominated in all sites, with alternative alleles occurring at frequencies < 0.1 at many sites (Table 1). Only the *Idh1* locus was consistently polymorphic, with the less common $Idh1^{100}$ allele having frequencies of 0.112 to 0.404 among the 19 sites. Based on these three variable loci, the level of genetic subdivision across the island was small, with an overall F_{ST} of 0.023. Nevertheless, although the subdivision was modest, it was statistically significant ($P < 0.01$) for each of the three loci, indicating that populations are locally independent.

Table 1

Allelic frequencies at variable gene loci in samples of *Rhagada* species from Barrow Island. N = sample sizes for *Gpi*, *Idh1* and *Pgm1*; for other loci, sample sizes were four individuals. The nine invariant loci examined are not shown.

		Gpi			*Idh1*		*Ldh*		*Pep-vl*		*Pgd*		*Pgm1*			
Site	N	*152*	*100*	*52*	*100*	*91*	*111*	*100*	*100*	*95*	*100*	*67*	*144*	*111*	*100*	*78*
Large species																
L01	33	0.045	0.894	0.061	0.303	0.697	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L02	40	0.025	0.975	...	0.138	0.863	1.000	...	1.000	...	1.000	...	0.062	...	0.938	...
L03	40	...	1.000	...	0.262	0.738	1.000	...	1.000	...	1.000	...	0.025	...	0.975	...
L04	40	0.013	0.913	0.075	0.363	0.637	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L05	48	...	0.938	0.062	0.229	0.771	1.000	...	1.000	...	1.000	...	0.010	...	0.990	...
L06	40	...	1.000	...	0.389	0.611	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L07	30	...	1.000	...	0.350	0.650	1.000	...	1.000	...	1.000	...	0.017	...	0.983	...
L08	40	...	0.988	0.013	0.112	0.887	1.000	...	1.000	...	1.000	...	0.025	...	0.975	...
L09	40	...	0.925	0.075	0.375	0.625	1.000	...	1.000	...	1.000	...	0.025	...	0.975	...
L10	36	...	0.972	0.028	0.278	0.722	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L11	40	...	0.925	0.075	0.262	0.738	1.000	...	1.000	...	1.000	...	0.062	...	0.938	...
L12	48	...	0.969	0.031	0.302	0.698	1.000	...	1.000	...	1.000	...	0.083	...	0.917	...
L13	40	...	0.950	0.050	0.325	0.675	1.000	...	1.000	...	1.000	...	0.062	...	0.938	...
L14	11	...	0.955	0.045	0.227	0.773	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L15	11	0.045	0.864	0.091	0.136	0.864	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L16	10	...	0.950	0.050	0.150	0.850	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L17	10	...	1.000	...	0.300	0.700	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L18	52	0.048	0.952	...	0.404	0.596	1.000	...	1.000	...	1.000	...	...	...	1.000	...
L19	10	...	0.950	0.050	0.200	0.800	1.000	...	1.000	...	1.000	...	...	...	1.000	...
Small species																
S20	4	0.500	0.500	...	...	1.000	...	1.000	0.500	0.500	...	1.000	...	1.000	...	...
S21	4	1.000	...	...	...	1.000	...	1.000	0.375	0.625	...	1.000	...	0.875	...	0.125

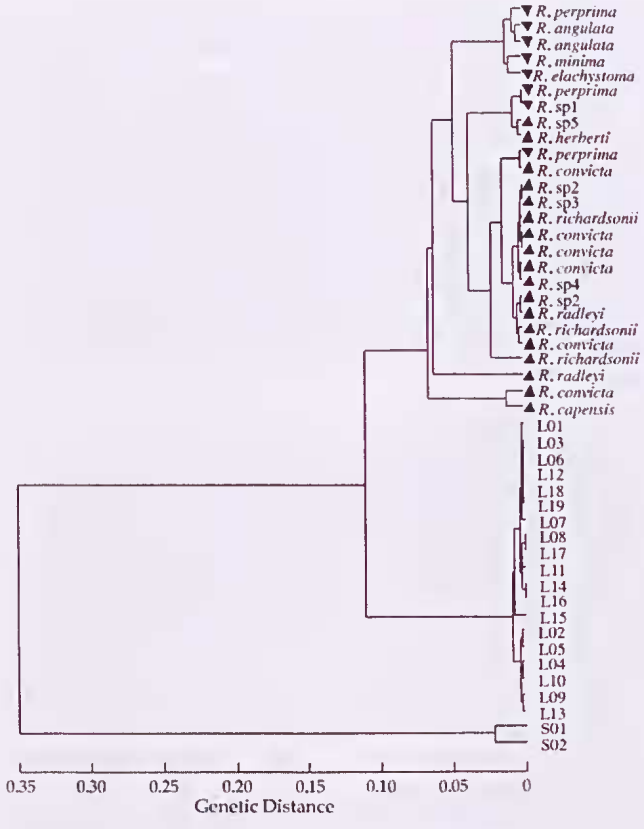

Figure 3. UPGMA phenogram of genetic distances among populations of the two species of *Rhagada* on Barrow Island and species on the Pilbara mainland (upright triangles) and Dampier Archipelago (inverted triangles). Site codes for Barrow Island as in Fig. 1 (small species = S01 to S02; large species = L01 to L19)

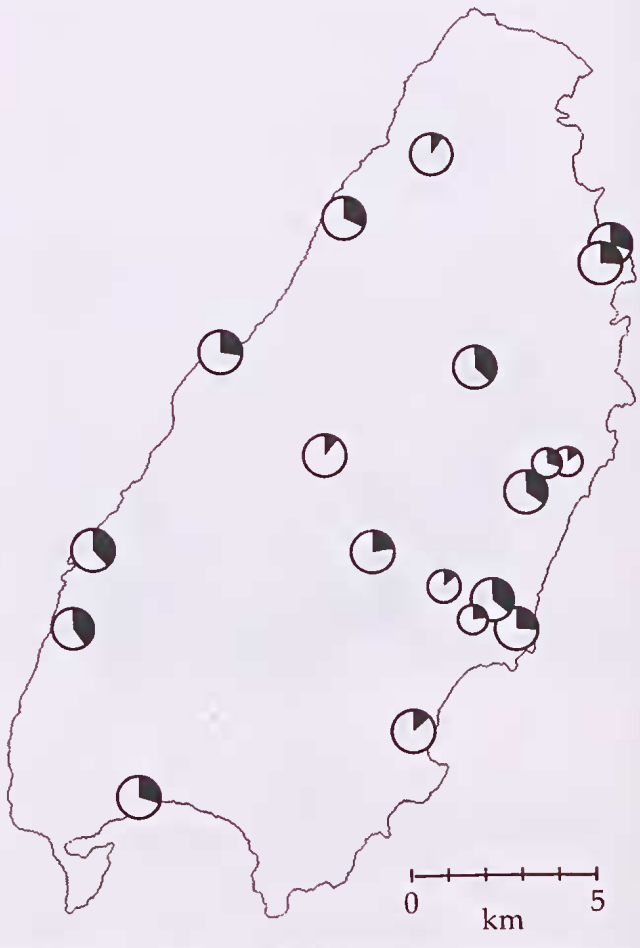

Figure 4. Frequencies of the $Idh1^{100}$ (shaded segments) and $Idh1^{91}$ (white segments) alleles in samples of the large species of *Rhagada*. Small circles = small samples.

Allelic frequencies at the *Idh1* locus showed no obvious geographic pattern across the island (Fig. 4). Similarly, the rarer alleles at the less polymorphic *Gpi* and *Pgm1* loci were scattered across the island (Table 1). Consistent with this apparent lack of geographic pattern, there was also no association of genetic divergence and geographic distance across the island ($r = 0.01$, $P > 0.8$, Mantel test). This analysis included the five small samples, as well as the 14 large samples, but removal of the small samples had no effect on the search for spatial pattern.

Discussion

These genetic comparisons confirm the occurrence of two morphologically and genetically distinct species of *Rhagada* on Barrow Island. Co-occurrence of species of *Rhagada* is extremely rare (Solem 1997; Johnson *et al.* 2004). The two species on Barrow Island fit this general pattern, with complementary distributions. These distributions reflect the distribution of contrasting habitats on the island. The northern end of the island, occupied by the small species, has a mosaic of *Triodia* species (Buckely 1983), and the smaller species of *Rhagada* was collected from flat hummock grasslands, whereas the widespread, larger species was found predominantly in and near ravines dominated by *Triodia angusta*, which are common outside the northern end of the island. Because the two species were not found together, we do not have direct evidence of intrinsic reproductive isolation. Nevertheless, the species were found within 0.8 km of each other, and the genetic differences were consistent throughout the distributions of both types, confirming that they are separate species.

These are the two most genetically divergent species of *Rhagada* so far examined in the Pilbara Region. Indeed, comparisons amongst all species of *Rhagada* on the Pilbara mainland did not reveal unique alleles in any of them (Johnson *et al.* 2004). In addition to highlighting the conservation significance of these endemic snails on Barrow Island, the genetic distinctiveness of the two species raises questions about the evolutionary radiation of *Rhagada*. The large species on Barrow Island is apparently closely related to the species on the Pilbara mainland and the Dampier Archipelago. In contrast, the small species apparently represents a distinct lineage, with no counterparts yet identified on the mainland. Its shells fit Solem's (1997) description of *R. plicata* on the nearby Montebello Islands, a species that has not yet been examined genetically. Although not recorded by Solem (1997), a large species, similar to that on Barrow Island, also occurs in the Montebellos (P. Kendrick, personal communication). Although the shells of the large species are very similar to those of several mainland species, the small species has shells unlike any described species from the Pilbara mainland. Instead, they are similar in

shape and sculpture to the shells of *R. dringi*, from 80-mile Beach. This raises the possibility of two lineages from the north leading to the colonization of Barrow Island and the Montebello Islands, only one of which diversified in the mainland Pilbara Region. More extensive phylogenetic analyses, using more sensitive molecular markers are needed to test this hypothesis.

In contrast with the substantial genetic divergence between the two species of *Rhagada* on Barrow Island, little genetic divergence was found among populations of the widespread, large species. The small genetic differences were statistically significant, however, indicating that populations are locally independent, as is typical of land snails. On the mainland, for example, demographically independent populations of *R. capensis* span less than 40 m (Johnson & Black 1991), well below the scale of resolution of the present study.

Despite independence of local populations, the level of genetic subdivision of the large species on Barrow Island over distances of up to 20 km is exceptionally low for a land snail (*e.g.*, Johnson 1976; Johnson *et al.* 1993; Davison & Clarke, 2000; Arnaud *et al.*, 2003). The frequencies of the common alleles at the *Idh1* locus show only modest variation across the island, and even the relatively uncommon alleles at the *Gpi* and *Pgm1* loci are widespread and are not restricted to particular portions of the island. Although additional genetic markers would be desirable, the genetic patterns within the large species of *Rhagada* on Barrow Island give no indication of geographic areas with genetically distinct populations. The low level of subdivision, combined with the lack of clear spatial pattern, suggests at least occasional gene flow across the whole island, perhaps facilitated by extreme wet periods during cyclonic rain.

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